

**PATOLOGIE ENDOCRINE
E CHIRURGIA:
INNOVAZIONI TECNOLOGICHE
E TRATTAMENTI MINI-INVASIVI**
Tiroide Paratiroidi Surreni Pancreas

Il percorso diagnostico del nodulo tiroideo: il ruolo dell'analisi molecolare

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Università degli Studi di Ferrara





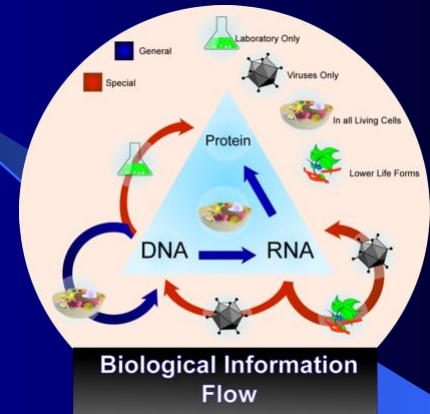
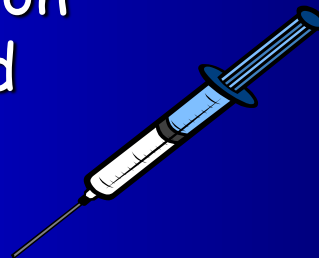
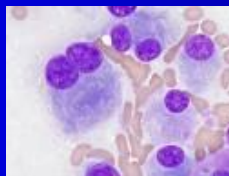
L'analisi molecolare nel nodulo tiroideo



MOLECULAR BIOLOGY
study of **biology** at molecular level

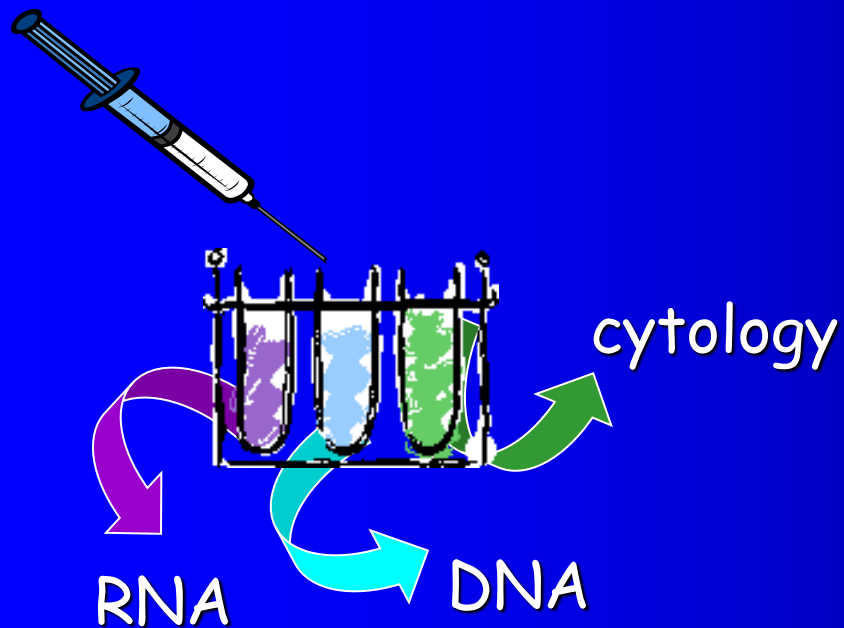
pathology

Does it improve cytological
fine needle aspiration
diagnosis of thyroid
nodules?





L'analisi molecolare nel nodulo tiroideo



rearrangement studies

somatic mutation analysis

15-20% FNAB inconclusive or unable to discriminate between follicular adenoma and carcinoma

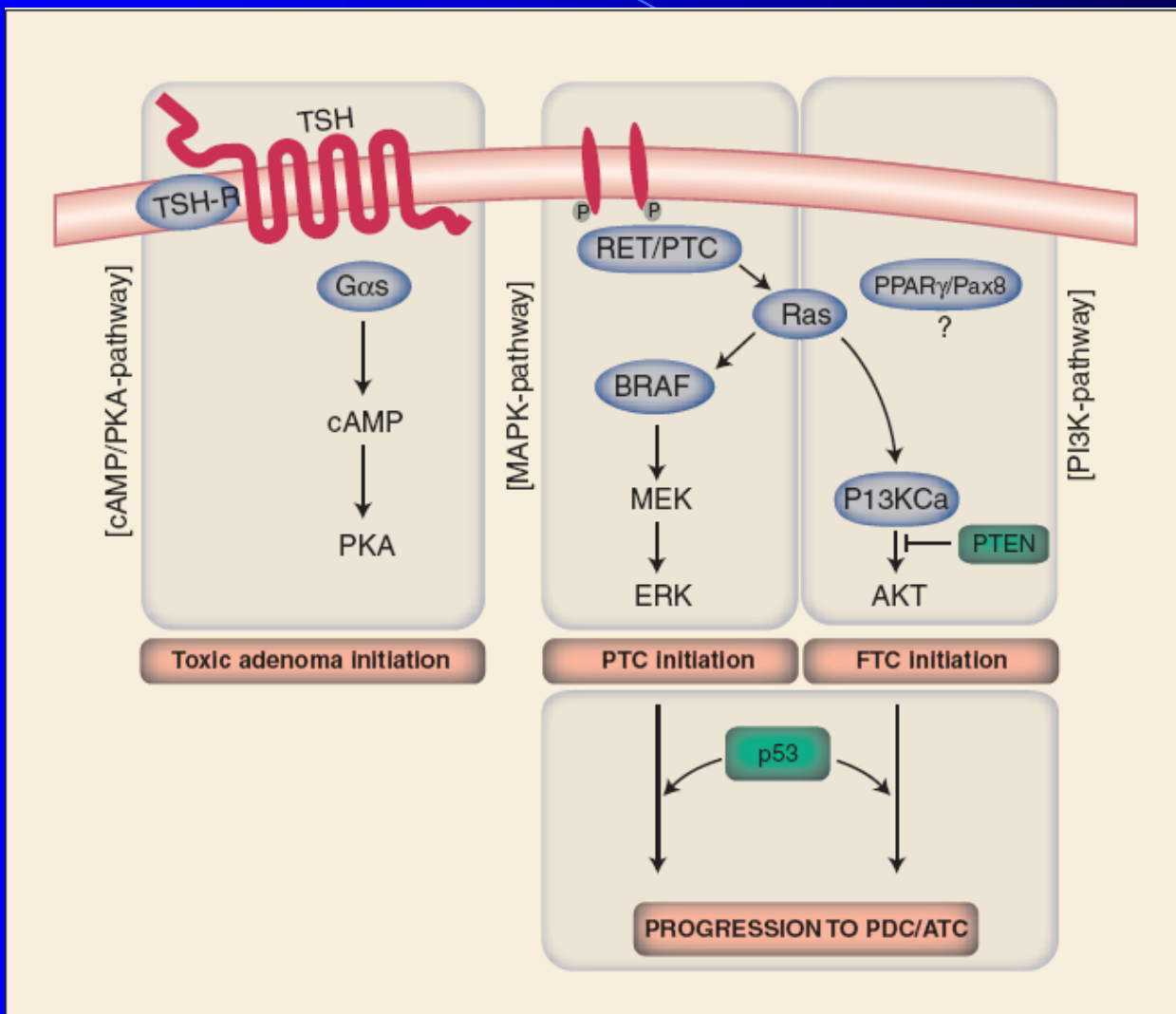
need for partial or total thyroidectomy for diagnostic purposes

Riesco-Eizaguirre et al. Clin Transl Oncol 2007, 9:686-693





L'analisi molecolare nel nodulo tiroideo

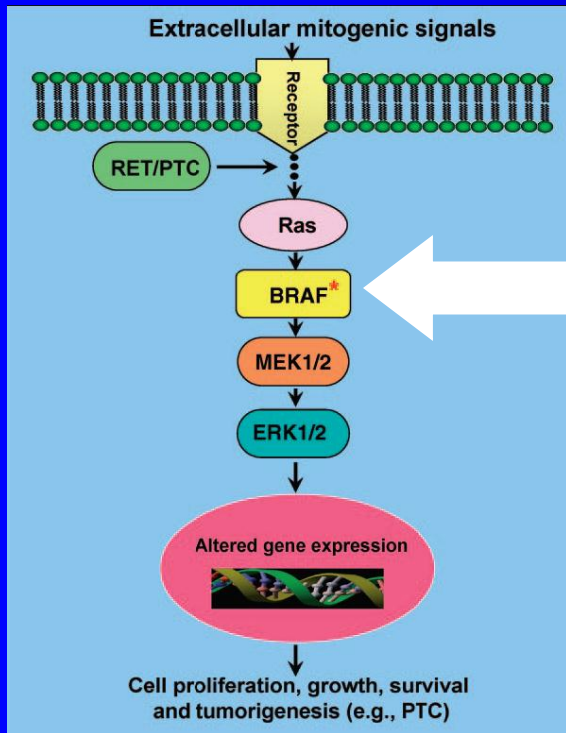




L'analisi molecolare nel nodulo tiroideo



PAPILLARY CARCINOMA

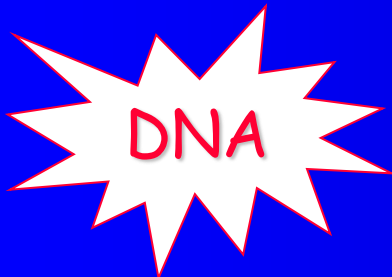


BRAF V600E point mutation

[K601E and V599Ins]

- ✓ 45-80% of PTC, mainly tall cell and classic hystology
- ✓ ↑ extrathyroidal invasion
- ✓ higher stage
- ✓ ↑ recurrence (with reduced I up-take)
- ✓ ↑ de-differentiation

Lupi et al. J Clin Endocrinol Metab. 2007;92:4085



restricted to PTC





L'analisi molecolare nel nodulo tiroideo



**BRAFV600E
molecular test**

somatic mutation analysis

pyrosequencing

Kim et al. J Clin Endocrinol
Metab, 2011, 96:658

MASA

Pelizzo et al. Clin Chem
Lab Med. 2011;49:325

RFLP

Zatelli et al. Eur J
Endocrinol 2009, 161:467

direct sequencing

Zatelli et al. Eur J
Endocrinol 2009, 161:467

allelic discrimination

Rossi et al. J Clin Endocrinol
Metab 2012;97:2354

**specific colorimetric mutation detection assay
(Mutector; TrimGen, Sparks, MD)**

Xing et al. J Clin Oncol. 2009;27:2977-82

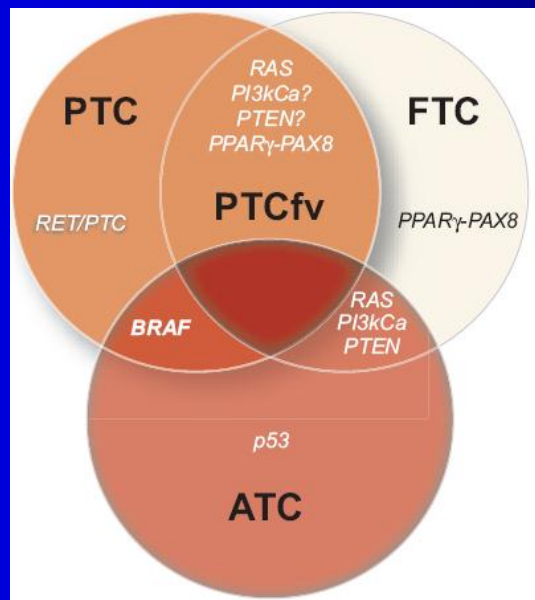


Affordable costs
Dedicated instruments
Experienced personnel





- does it improve diagnosis?



L'analisi molecolare nel nodulo tiroideo



BRAFV600E molecular test



	Cytology		BRAF		Cytology + BRAF	
	100	77.3	89.6	64.0	89.6	86.7
Sensitivity	100	77.3	89.6	64.0	89.6	86.7
Specificity	36.4	98.8	95.5	100	95.5	98.8
PPV	92.9	92.1	99.4	100	99.4	92.9
NPV	100	95.9	52.5	93.7	52.5	97.5
Accuracy	93.3	95.4	90.2	95.4	90.2	96.9
K value	0.51±0.11	0.81±0.02	0.63±0.07	0.76±0.05	0.63±0.07	0.88±0.01



Kim et al. J Clin Endocrinol Metab, 2011, 96:658

Zatelli et al. Eur J Endocrinol 2009, 161:467

EFE 2012





L'analisi molecolare nel nodulo tiroideo

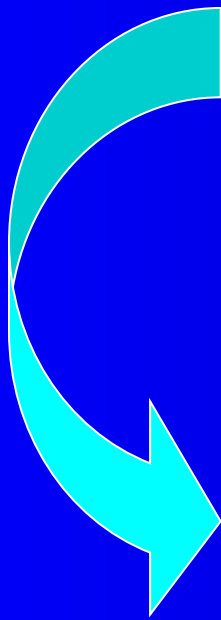


**BRAFV600E
molecular test**



	BRAF ^{V600E} (+)	BRAF ^{V600E} (-)	<i>P</i> value
n	154 (91.1)	15 (8.9)	
Age at diagnosis (yr)	48.8 ± 12.1	40.1 ± 12.7	0.01
<45	56 (36.4)	8 (53.3)	0.31
≥45	98 (63.6)	7 (46.7)	
Gender			
Male	22 (14.3)	2 (13.3)	0.77
Female	132 (85.7)	13 (86.7)	
Tumor size (mm)	10.90 ± 9.63	11.07 ± 5.78	0.95
<5	26 (16.9)	3 (20.0)	0.02
5 to <10	74 (48.1)	2 (13.3)	
10 to <15	19 (12.3)	6 (40.0)	
15 to <20	17 (11.0)	3 (20.0)	
≥20	18 (11.7)	1 (6.7)	
Extrathyroidal invasion	83 (53.9)	6 (40.0)	0.44
Lymph node metastasis	55 (35.7)	4 (26.7)	0.67

Kim et al. J Clin Endocrinol Metab, 2011, 96:658



**BRAF mutation analysis
may help especially in
small nodules**





L'analisi molecolare nel nodulo tiroideo



US and clinical findings suspected for malignancy

US findings

Hypoechoic

Isoechoic

Hyperechoic

Microcalcifications

Blurred margins

Intranodular
vascularity

Clinical findings

Age (<30 or > 60 years)

Male gender





L'analisi molecolare nel nodulo tiroideo



Section of Endocrinology
University of Ferrara

1856 pz

(1436 ♀; 420 ♂)

age 52 ± 0,32 (12-73)

US & clinical evaluation

2421 FNAs

Biomolecular analysis

Pathology

All nodules $\varnothing \geq 4$ mm
with or without clinical/US
characteristics suspected
for malignancy





L'analisi molecolare nel nodulo tiroideo



US findings	Total (2421)	Cancers (233)
Hypoechoic	1173	168
< 1 cm	749	89
> 1 cm	429	79
Isoechoic	1010	38
< 1 cm	341	20
> 1 cm	669	18
Hyperchoic	97	6
< 1 cm	51	1
> 1 cm	46	5
Microcalcifications	675	107
< 1 cm	310	46
> 1 cm	365	61
Blurred margins	62	27
< 1 cm	32	8
> 1 cm	30	19
Intranodular vascularity	84	12
< 1 cm	32	4
> 1 cm	52	8

Clinical findings	Total (2421)	Cancers (233)
Age (<30 or > 60 years)	861	85
< 1 cm	382	32
> 1 cm	479	58
Male gender	514	43
< 1 cm	234	27
> 1 cm	280	16



All clinical/US characteristics suspected for malignancy were investigated



L'analisi molecolare nel nodulo tiroideo



Number of clinical/US findings suspected for malignancy in nodules diagnosed as cancer at histology	
None	4
< 1 cm	3
> 1 cm	1
One	59
< 1 cm	41
> 1 cm	18
Two	79
< 1 cm	62
> 1 cm	17
More than two	91
< 1 cm	34
> 1 cm	57



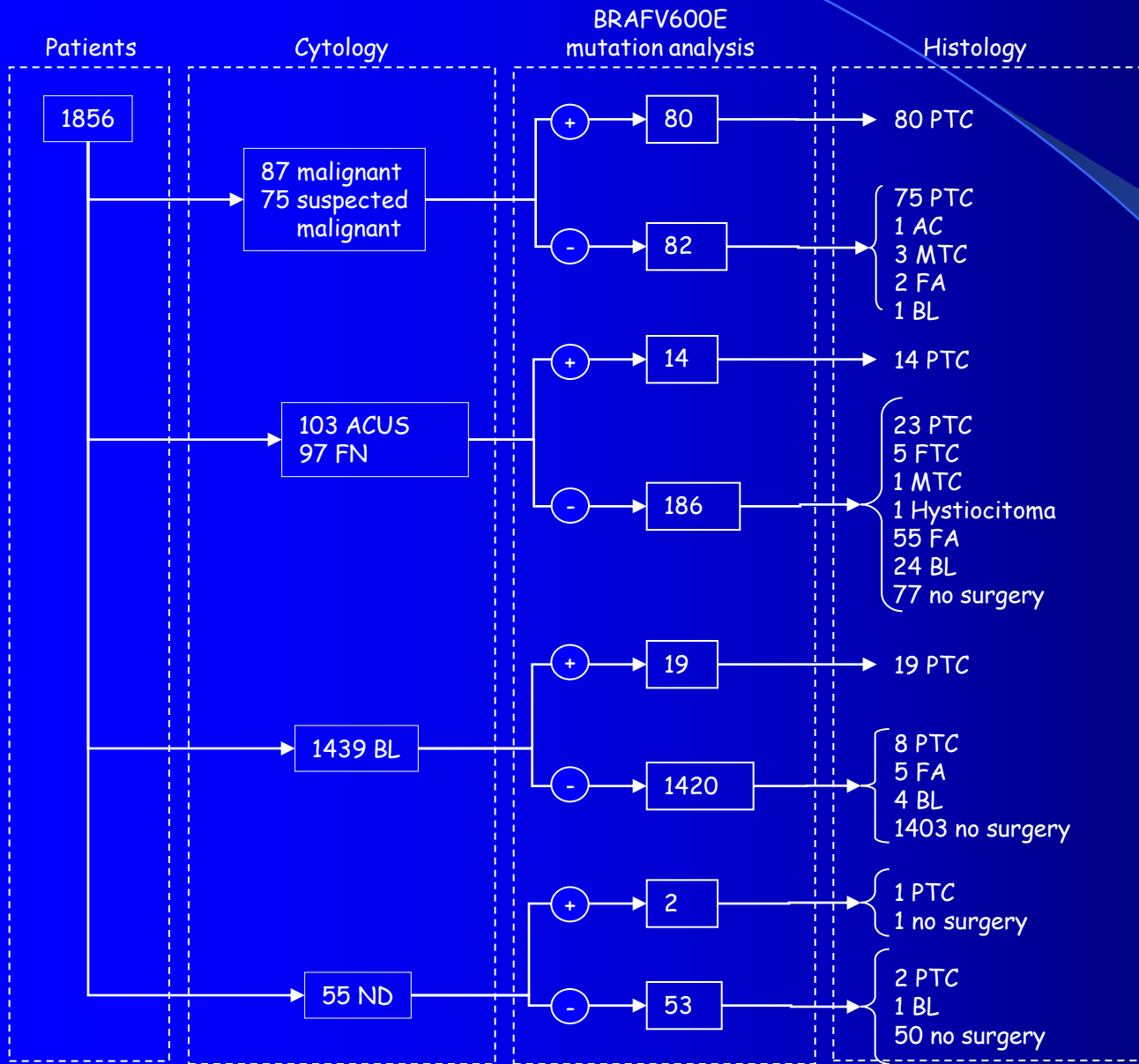
Even nodules lacking clinical/US findings suspected for malignancy may underlie a thyroid cancer!!!

None of the clinical/US findings suspected for malignancy predict BRAF status





L'analisi molecolare nel nodulo tiroideo



233 thyroid carcinomas

Rossi et al JCEM 2012;97:2354

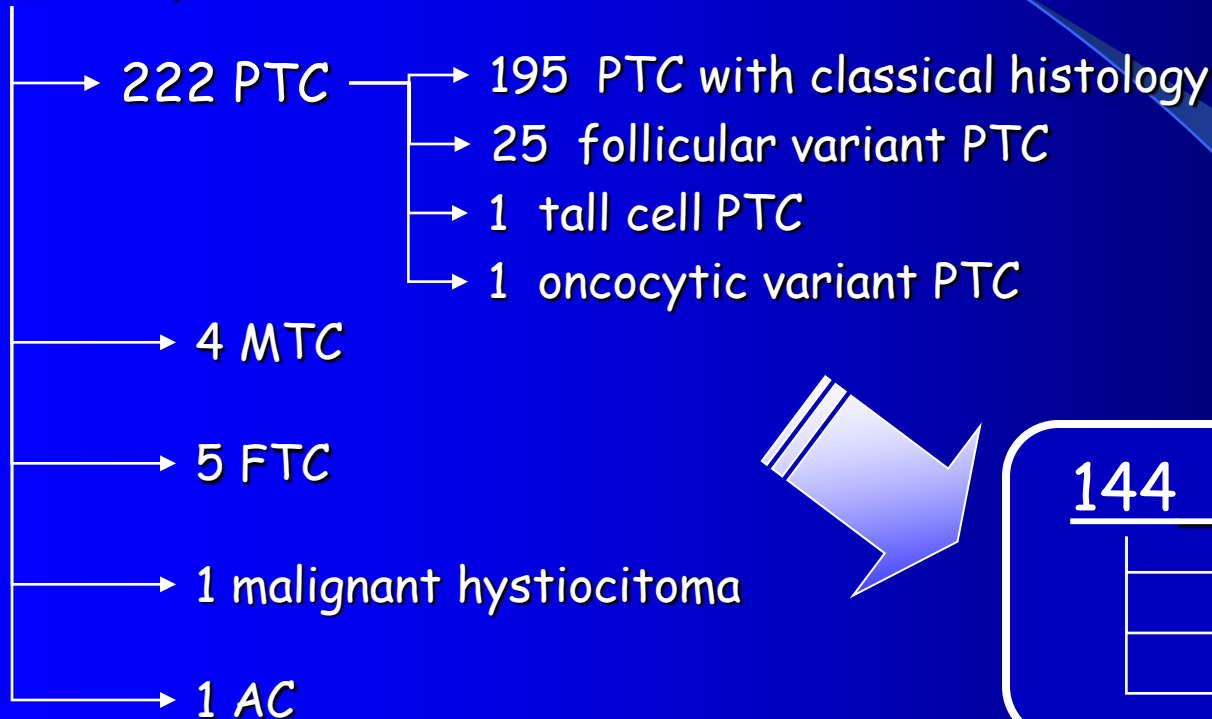




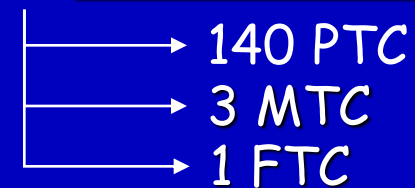
L'analisi molecolare nel nodulo tiroideo



233 thyroid carcinomas



144 microcarcinomas





L'analisi molecolare nel nodulo tiroideo



Cancer prevalence

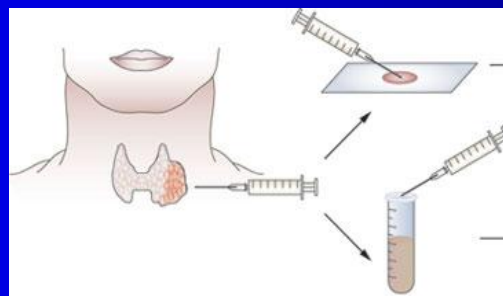
nodules >1 cm	nodules <1 cm
7.2%	12.2%

$p < 0.001$

140 microPTC

- 13 multifocal
- 17 with lymphnode metastases

All nodules should undergo FNAB





L'analisi molecolare nel nodulo tiroideo



BRAFV600E molecular test	Cytology		BRAF		Cytology + BRAF	
	S	NS	S	NS	S	NS
Sensitivity	76,8	69,4	56,6	51	92,9	84,7
Specificity	99,7	99,9	100	100	99,7	99,9
PPV	97,7	98,6	100	100	98,1	98,8
NPV	96,5	98	93,6	96,9	98,9	99
Accuracy	96,6	98,1	94,1	97	98,8	99

BRAF testing significantly increases FNAB sensitivity also in nodules clinically non suspected

15 PTC patients "rescued" by BRAF analysis





L'analisi molecolare nel nodulo tiroideo



BRAFV600E molecular test	S	%	NS	%
ACUS	26	3,1	78	4,9
PTC	6	75,0	10	37,0
BRAF +	5	19,2	0	0,0
FN	35	4,2	62	1,2
PTC	5	25,0	9	23,7
BRAF +	2	5,7	6	9,7



BRAF testing identifies
as malignant 10% of FN

Indication to total
thyroidectomy





L'analisi molecolare nel nodulo tiroideo



CONCLUSION -1



BRAF molecular analysis increases
diagnostic sensitivity of cytology for PTC
and
influences clinical management





L'analisi molecolare nel nodulo tiroideo



- any prognostic value?





L'analisi molecolare nel nodulo tiroideo



PAPILLARY CARCINOMA

BRAF mutation(s)

- ⊖ → NIS expression
- ⊖ → NIS trafficking to the membrane
Riesco-Eizaguirre et al. Endocrine-Related Cancer 2006, 13: 257
- ⊕ → DNA synthesis and apoptosis



⊕

MMP, vimentin, osteopontin

epithelial-mesenchymal transition

little growth advantage

BUT

genomic instability

Mitsutake et al. Cancer Research 2005;65: 2465



L'analisi molecolare nel nodulo tiroideo



BRAFV600E molecular test



Table 1. Association of *BRAF* Mutation Status Detected on Thyroid Fine-Needle Aspiration Biopsy With Poorer Clinicopathologic Characteristics of Papillary Thyroid Cancer

Characteristic	<i>BRAF</i> Positive (n = 73)		<i>BRAF</i> Negative (n = 117)		P*
	No.	%	No.	%	
Age at diagnosis, years					.28
Median	43		46		
Range	24-77		12-83		
Sex, male	24	32.9	29	24.8	.25
Tumor size, cm					.35
Median	1.8		1.5		
Range	0.6-10.0		0.6-6.0		
Extrathyroidal extension	17	23.3	13	11.1	.039
Capsular invasion	21	28.8	19	16.2	.045
Lymph node metastasis	28	38.4	21	18.0	.002
I	54	74.0	89	76.1	
II	4	5.5	15	12.8	
III	9	12.3	9	7.7	
IV	6	8.2	4	3.4	
III/IV	15	20.6	13	11.1	.093
Multifocality	34	46.6	47	40.2	.45

Abbreviation: AJCC, American Joint Committee on Cancer.

*P value from Fisher's exact test for categorical data and Wilcoxon rank sum test for continuous data.





L'analisi molecolare nel nodulo tiroideo



BRAFV600E molecular test	PTC persistence/recurrence prediction	
	All PTC	Conventional PTC
sensitivity	68%	79%
specificity	66%	60%
PPV	36%	34%
NPV	88%	92%

Xing et al. J Clin Oncol 2009; 27:2977-82

BRAF mutation-positive patients are significantly more likely to have PTC persistence/recurrence



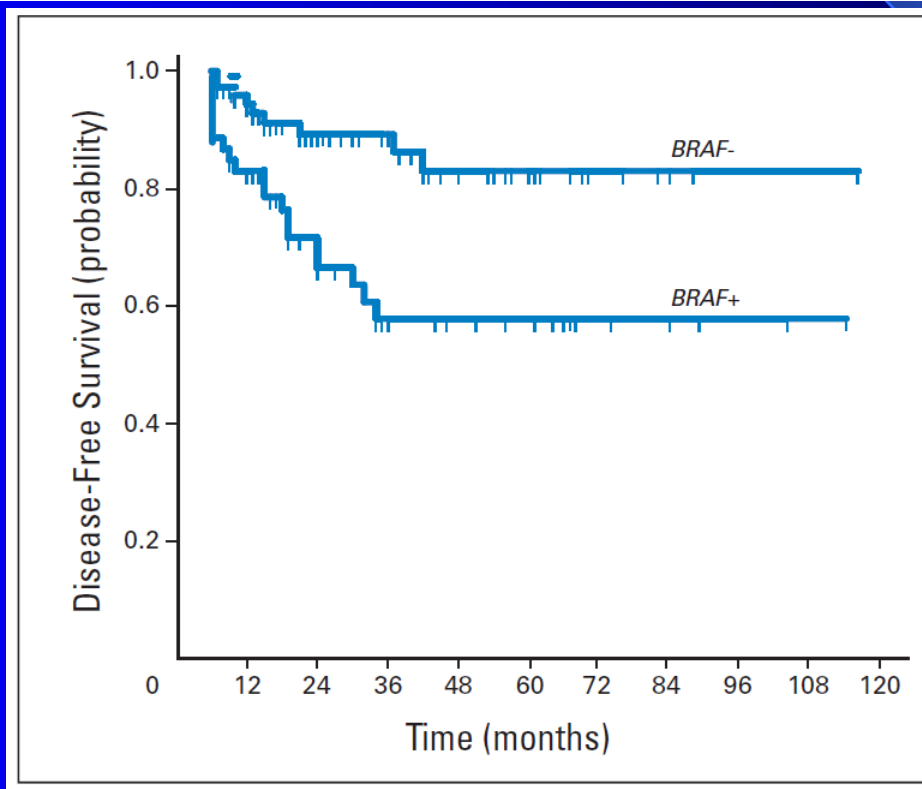


L'analisi molecolare nel nodulo tiroideo



**BRAFV600E
molecular test**

**significantly reduced disease-free probability
in BRAF+ patients**



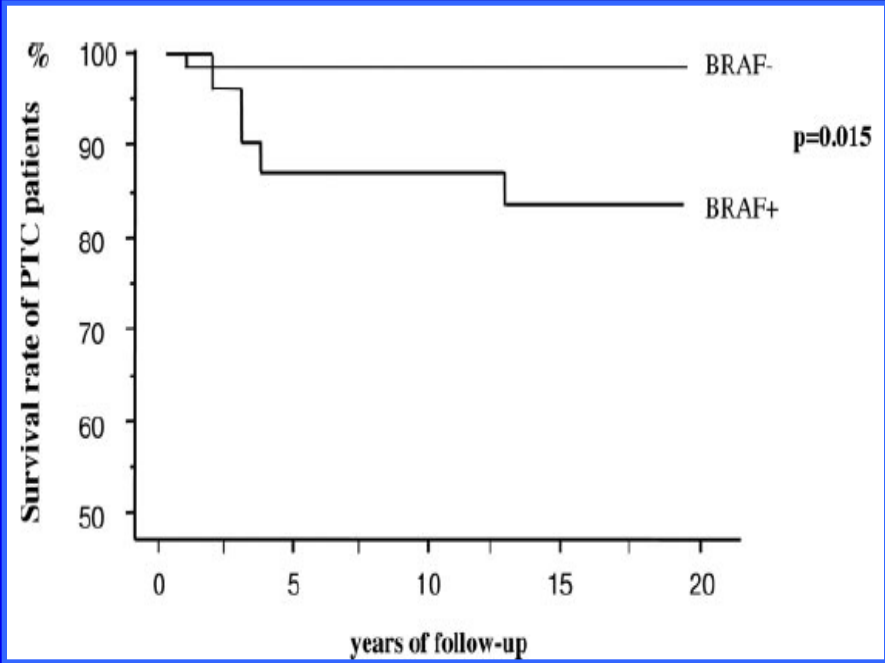


L'analisi molecolare nel nodulo tiroideo



**BRAFV600E
molecular test**

**significantly increased mortality
in BRAF+ patients**



Elisei et al. J Clin Endocrinol Metab. 2008;93:3943





CONCLUSION -2

BRAF status may predict patients outcome





L'analisi molecolare nel nodulo tiroideo



- any surgical relevance ?



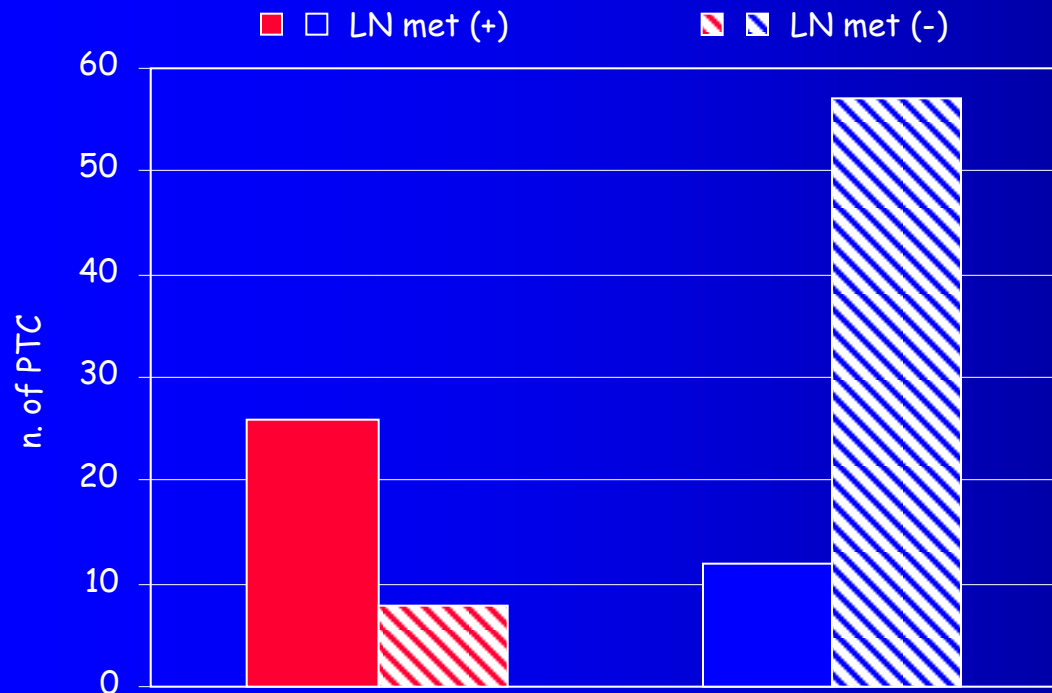


L'analisi molecolare nel nodulo tiroideo



BRAFV600E molecular test

103 PTC



BRAF (+)
34

BRAF (-)
69

20/26 BRAF+ LN

lymph node metastases
may show *de novo*
mutations

→ is not necessary for
locoregional lymph node
metastasis

→ is a significant risk
factor for locoregional
lymph node metastasis

Vasko et al. J Clin Endocrinol Metab 2005; 90: 5265

!! also in microPTC !!

Lin et al. Ann Surg Oncol 2010;17:3294

Kim et al. Ann Surg 2006;244: 799

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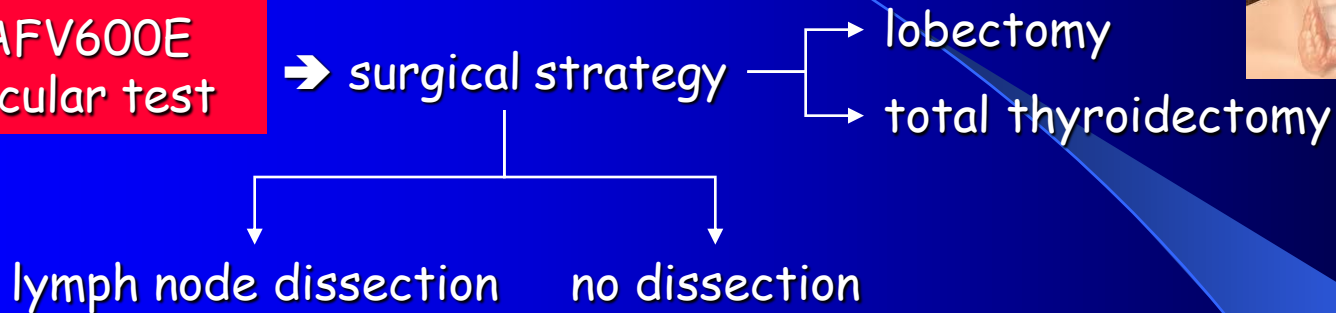




L'analisi molecolare nel nodulo tiroideo



**BRAFV600E
molecular test**



better staging

High prognostic impact

extrathyroidal invasion
lymph node metastasis
local neck recurrence
PTC recurrence
complications

**what
lymphnode
dissection?**

Xing Endocrine Reviews 2007; 28: 742





L'analisi molecolare nel nodulo tiroideo



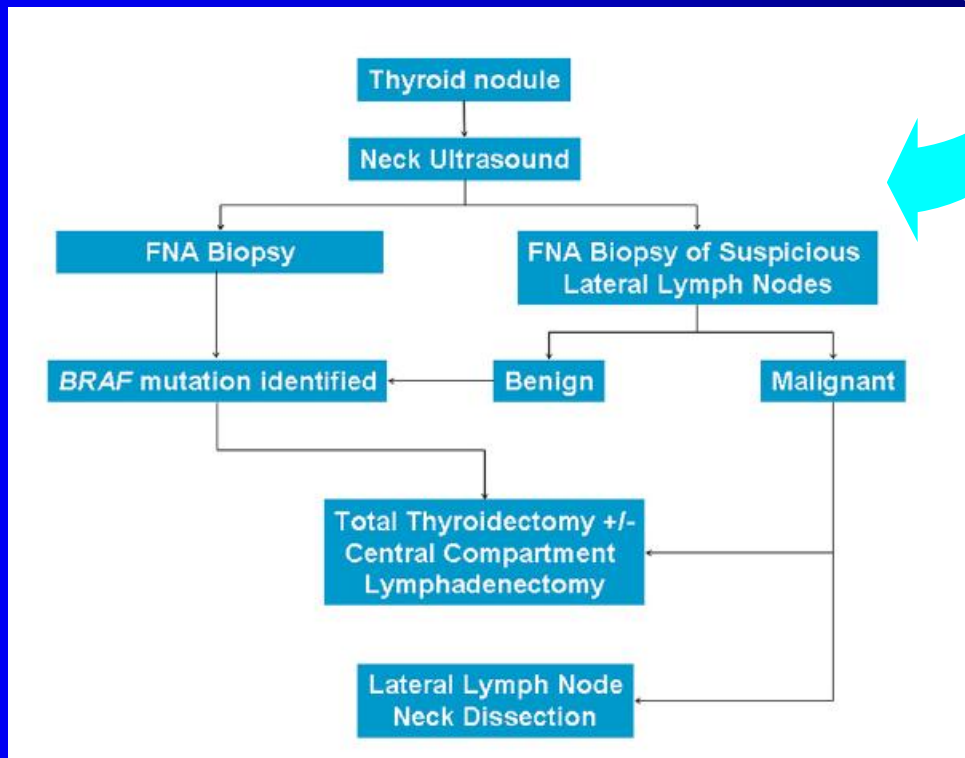
**BRAFV600E
molecular test**



PREDICTOR OF LYMPHNODE METASTASES



Indication for sentinel lymphnode





CONCLUSION -3

BRAF V600E may influence
surgical approach





L'analisi molecolare nel nodulo tiroideo



**BRAFV600E
molecular test**

May address patients with
persistent/recurrent disease
to therapy with
BRAF-specific inhibitors



Cantwell-Dorris et al. Mol Cancer Ther 2011, 10: 385

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L'analisi molecolare nel nodulo tiroideo

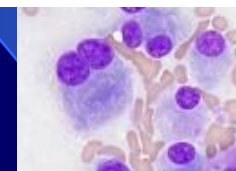


THEREFORE



**BRAFV600E
molecular test**

FNAB material



- ✓ increases cytology diagnostic sensitivity for PTC
- ✓ may predict patients outcome
- ✓ influences surgical approach
- ✓ allows detection of minimal disease metastatic to cervical lymph nodes

Xing et al. 2004 J Clin Endocrinol Metab 89:2867
Cohen et al. 2004 Clin Cancer Res 10:2761
Domingues et al. 2005 Cytopathology 16:27
Zatelli et al 2009 J Clin Endocrinol Metab
Nikiforov et al. 2009 J Clin Endocrinol Metab 94:2092
Rossi et al 2012 Clin Endocrinol Metab
Kim et al. 2006 Ann Surg 244:799

Kim et al. 2006 Clin Endocrinol 65:364
Xing 2007 Endocr Rev 28:742
Nikiforova et al 2008 Expert Rev Mol Diagn 8:83
Riesco-Eizaguirre et al. 2006. Endocr Rel Cancer 13:257
Xing et al. 2005 J Clin Endocrinol Metab 90:6373
Mojica et al 2006 Endocr Pathol 17:183





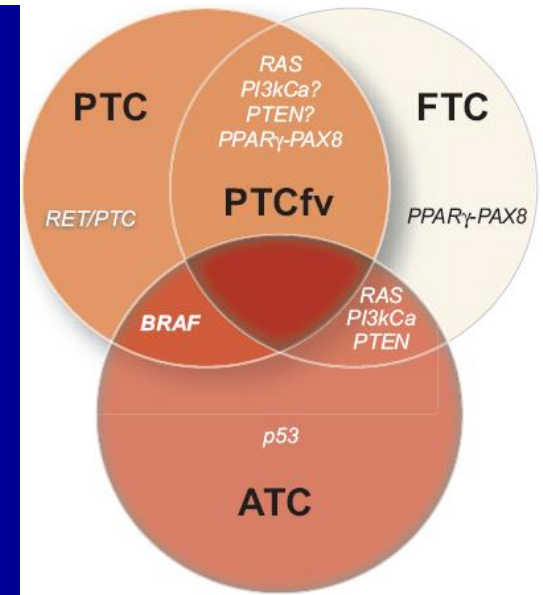
L'analisi molecolare nel nodulo tiroideo



	BRAF	RET/PTC	RAS	PI3KCa*	PTEN	PPAR γ /PAX8
PTC	29–83%	2.5–59%**	–	–	–	–
PTCfv	–	–	5–15%	15%	2%	37.5%
FTC	–	–	7–62%	8–42%	6–7%	36–45%
FA	–	14%	9–11%	8–23%	–	4–33%
ATC/PDC	10–35%	–	50–55%	54%	16%	–
Extrathyroid extension	Yes	No	–	–	–	–
Increased recurrence risk	Yes	No	–	–	–	–
Poor survival	?	No	Yes	–	–	–

Riesco-Eizaguirre et al. Clin Transl Oncol 2007; 9:686

molecular biology can help





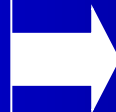
L'analisi molecolare nel nodulo tiroideo



BUT

Molecular testing
is not sufficient
to detect all malignant cases

30% PTC
20% FTC
>50% oncocytic FTC



no known mutations!!!

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83



THANKS

Section of Endocrinology
Dept. of Biomedical Sciences and Advanced Therapies
University of Ferrara
Ettore degli Uberti

